PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
ALLEMAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 12.04.2001
Applicant's or agent's file reference C 2781 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00554	25/01/2000	25/01/1999

Applicant
BIOCHIP TECHNOLOGIES GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Digiusto, M Tel.+49 89 2399-8162

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 2781 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00554	25/01/2000	25/01/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant BIOCHIP TECHNOLOGIES GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/06/2000	12.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moreno de Vega, C Telephone No. +49 89 2399 7486

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00554

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-21	as originally filed				

Claims, No.:

1-23	as received on	12/01/2001	with letter of	11/01/2001	

Drawings, sheets:

1/1	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 6, 10-14
	No:	Claims 1-5, 7-9, 15-23
Inventive step (IS)	Yes:	Claims 11-14
	No:	Claims 1-10, 15-23
Industrial applicability (IA)	Yes:	Claims 1-23
	No:	Claims

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 97 41425 A (PENCE INC ;UNIV MCGILL (CA)) 6 November 1997 (1997-11-06)

D2: WO 90 05303 A (PHARMACIA AB) 17 May 1990 (1990-05-17)

D3: SÖNKSEN C.P. ET AL.: 'Combining Maldi mass spectrometry and biomolecular interaction analysis using a biomolecular interaction analysis instrument' ANAL. CHEM., vol. 70, - 1 July 1998 (1998-07-01) pages 2731-2736

The International Examining Authority in charge of this application has considered the applicant's arguments in response to the Written Opinion, nevertheless the following objections are still maintained. Furthermore, the feature "obtainable by the process comprising the steps of...a)... b)..." in claim 1 is not limiting the scope of the claim (see further Item VIII).

1. Novelty (Article 33 (2) PCT)

1.1 Claims 1-5, 7-9, 15-23 are not considered to be novel, as they are already disclosed in the prior art.

D1 (see page 4 lines 5-18, example 2, claims and figures) discloses a biosensor apparatus for detecting a binding event between a ligand and a receptor. The biosensor includes an electrode substrate coated with a high-dielectric hydrocarbon-chain monolayer, having ligands attached to the exposed monolayer surface and measuring the binding of a receptor to the monolayer-bound ligand. The hydrocarbon chains are attached via a thiol group. This document appears to be novelty destroying for claims 1-5, 7-9, 15-23.

D2 (see especially pages 6-8, claims) discloses methods for the production, on metal surfaces, of surface layers which are capable of selective biomolecular interactions, sensing surfaces produced by means of these methods and the use thereof in biosensors. The surface layers are

monolayers of organic molecules (e.g. hydrocarbon chains) attached by thiol, nitro, etc. groups and which contains active groups that bind ligands and biomolecules. This document appears to be novelty destroying for claims 1-5, 7-9, 19-21.
D3 (see especially page 2732) studies the interaction of binding of myoglobin to an immobilized (on a carboxymethyl-dextran/gold surface of a sensor chip) monoclonal IgG directed against human myoglobin and capturing of the DNA binding protein PaR by a biotinylated double-stranded (ds) DNA probe bound to the sensor chip via biotin-streptavidin interaction. This document appears to be novelty destroying for claims 1, 9, 16-21.

1.2 Claims 6, 10-14 are considered to be novel, as their subject-matter is not disclosed in the prior art.

2. Inventive step (Article 33(3) PCT)
Claims 6 and 10 differ from D1, which is considered to be the most relevant prior art, in that the polymer contains specific monomers which provide water swellability and in that the polymer chains are disposed on the surface in patterned arrays. The technical problem to be solved by these claims is the provision of improved surfaces useful as biosensors. D1 and D2 solve the same technical problem; claims 6 and 10 do not contain any feature which in combination with the features of the claims to which they refer, in the light of D1, D2 and the general knowledge in the field, meet the requirements of the PCT with respect to inventive step. Thus, claims 6 and 10 are not considered to be inventive.

Claims 11 to 14 differ from D2, considered to be the most relevant prior art for these claims, in the method of producing the polyfunctional polymer monolayer, i.e. said method comprises first covering the surface with a monolayer of polymerization initiator with groups suitable to attach it to the surface, and carrying out a polymerization in the presence of monomers with functional groups which allow the coupling. If the polymer obtained with the specific molecules. The technical problem to be solved by these claims is the provision of a method of producing improved polyfunctional polymer monolayers useful in the detection of biological molecules. There is not hint

in the prior art to arrive at the solution proposed by these claims. Thus, claims 11-14 are considered to be inventive.

Re Item VII
Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2 and D3 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

Claim 1 is not clear (Article 6 PCT), as it intends to define its subject-matter (product) in terms of the process used to obtain the product claimed.

PCT/EP00/00554
BIOCHIP TECHNOLOGIES GMBH et al.
Our Ref.: C 2781 PCT

Claims

1. Polyfunctional polymer monolayer comprising an assembly of polymer chains attached to a surface obtainable by a process comprising the steps of:

   a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

   b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules,

   wherein each polymer chain comprises a multitude of identical or different units carrying one or more functional groups which allow an interaction of the polymer with a sample or probe molecule.

2. Polymer monolayer according to claim 1, wherein the polymer chains are covalently attached to the surface.

3. Polymer monolayer according to claim 1 or 2, wherein the functional groups are chosen from carboxylic acids, maleinimides, N-hydroxy succinimides, epoxides, isothiocyanates, isocyanates or azides.

4. Polymer monolayer according to any of claims 1 to 3, wherein the sample molecule or probe molecule is chosen from proteins, peptides, polysaccharides or nucleic acids and derivatives thereof.

5. Polymer monolayer according to any of claims 1 to 4, wherein the polymer comprises segments that make the layer water swellable.

6. Polymer monolayer according to claim 5, wherein the water swellability is provided by monomers chosen from acrylic acid, methacrylic acid, dimethyl acrylamide or vinyl pyrrolidon.

7. Polymer monolayer according to any of claims 1 to 3, 5 and 6, further comprising a multitude of identical or different probe molecules immobilized at the polymer chain via a reaction with the functional groups.

8. Polymer monolayer according to claim 7, wherein the probe molecules are selected from nucleic acids, PNAs, polysaccharides, proteins and peptides.

9. Surface carrying a polyfunctional polymer monolayer according to any of claims 1 to 8.

10. Surface according to claim 9, wherein the polymer chains are in the form of patterned arrays.

11. Process for the production of a polyfunctional polymer monolayer according to any of claims 1 to 8, comprising the steps of:

    a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

    b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules.

12. Process according to claim 11, wherein the initiator comprises a chlorosilane, an alkoxysilane, a disulphide or a thiol group.

13. Process according to claims 11 or 12 wherein the initiator comprises a group chosen from azo groups, peroxo groups, or a ketone group in conjugation with an aromatic system.

14. Process according to claim 13, wherein the initiator comprises a group chosen from aromatic ketones or aromatic ketones containing sulphur.

15. Process for the detection of sample nucleic acid molecules, using a polymer monolayer according to claim 7 or 8, which comprises the steps of

    a) allowing a hybridization reaction to take place between the probe and the sample, followed by
    b) removal of the non hybridized nucleic acid molecules in a washing step and
    c) detection of the hybridized nucleic acid molecules.

16. A process for purifying a compound from a sample comprising the steps of

    (a) contacting the sample with the polymer monolayer of any of claims 1 to 8, under conditions that allow binding of said compound to the functional group of the polymer chain or the probe molecule;
    (b) and removing material from the sample that has not bound to the polymer layer or a probe molecule;

17. The process according to claim 16 further comprising

    (c) eluting the bound complex from the polymer layer.

18. The process according to claim 16 or 17, wherein said compound is a nucleic acid, a (poly)saccharide or a (poly)peptide or a complex thereof, preferably an antibody or a fragment or derivative thereof.

19. Use of the surface according to claims 9 or 10 as an affinity matrix.

20. Use of a surface according to claims 9 or 10 in a sensor chip.

21. Medical or diagnostic instrument, comprising a surface according to claims 9 or 10.

22. Use of a surface according to claims 9 or 10 for the immobilization of starter molecules for the formation of oligo- or polymers, preferably for nucleic acid or peptide synthesis.

23. Use of polymer layer according to any of claims 1 to 8 as a gel in the separation of molecules in an electric field.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
VOSSIUS & PARTNER
Siebertstrasse 4
81675 München
ALLEMAGNE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	12.09.2000
Applicant's or agent's file reference: C 2781 PCT	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00554	25/01/2000	25/01/1999

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
BIOCHIP TECHNOLOGIES GMBH et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 25/05/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Moreno de Vega, C

Formalities officer (incl. extension of time limits)
Digiusto, M
Telephone No. +49 89 2399 8162

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-21 as originally filed

Claims, No.:

1-23 as originally filed

Drawings, sheets:

1/1 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-5, 7-9, 11,12, 15-23
Inventive step (IS)	Claims	1-23
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 97 41425 A (PENCE INC ;UNIV MCGILL (CA)) 6 November 1997 (1997-11-06)

D2: WO 90 05303 A (PHARMACIA AB) 17 May 1990 (1990-05-17)

D3: SÖNKSEN C.P. ET AL.: 'Combining Maldi mass spectrometry and biomolecular interaction analysis using a biomolecular interaction analysis instrument' ANAL. CHEM., vol. 70, - 1 July 1998 (1998-07-01) pages 2731-2736

1. Novelty (Article 33 (2) PCT)

1.1 Claims 1-5, 7-9, 11-12, 15-23 are not considered to be novel, as they are already disclosed in the prior art.

D1 (see page 4 lines 5-18, example 2, claims and figures) discloses a biosensor apparatus for detecting a binding event between a ligand and a receptor. The biosensor includes an electrode substrate coated with a high-dielectric hydrocarbon-chain monolayer, having ligands attached to the exposed monolayer surface and measuring the binding of a receptor to the monolayer-bound ligand. The hydrocarbon chains are attached via a thiol group. This document appears to be novelty destroying for claims 1-5, 7-9, 11, 12, 15-23.

D2 (see especially pages 6-8, claims) disclosed methods for the production, on metal surfaces, of surface layers which are capable of selective biomolecular interactions, sensing surfaces produced by means of these methods and the use thereof in biosensors. The surface layers are monolayers of organic molecules (e.g. hydrocarbon chains) attached by thiol, nitro, etc. groups and which contains active groups that bind ligands and biomolecules. This document appears to be novelty destroying for claims 1-5, 7-9, 11, 12, 19-21.

D3 (see especially page 2732) studies the interaction of binding of myoglobin

to an immobilized (on a carboxymethyl-dextran/gold surface of a sensor chip) monoclonal IgG directed against human myoglobin and capturing of the DNA binding protein PaR by a biotinylated double-stranded (ds) DNA probe bound to the sensor chip via biotin-streptavidin interaction. This document appears to be novelty destroying for claims 1, 9, 16-21.

1.2 Claims 6, 10, 13 and 14 are considered to be novel, as their subject-matter is not disclosed in the prior art.

2. Inventive step (Article 33(3) PCT)
Claims 6, 10, 13 and 14 differ from D1, which is considered to be the most relevant prior art, in that the polymer contains specific monomers which provide water swellability, in that the polymer chains are disposed on the surface in patterned arrays, and in the specific polymerization initiators. The technical problem to be solved by these claims is the provision of improved surfaces useful as biosensors and of methods to produce the same. D1 and D2 solve the same technical problem; claims 6, 10, 13 and 14 do not contain any feature which in combination with the features of the claims to which they refer, and in the light of D1, D2 and the general knowledge in the field, meet the requirements of the PCT with respect to inventive step. Thus, claims 6, 10, 13 and 14 are not considered to be inventive.

Re Item VII
Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2 and D3 is not mentioned in the description, nor are these documents identified therein.

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Europäisches Patentamt | **European Patent Office** | **Office européen des brevets**

EPA/EPO/OEB - D-80298 München

Vossius & Partner
Sieberstrasse 4
81675 München

EINGEGANGEN
Vossius & Partner
28. JAN. 2000
Frist
oearb.:

Nr. der Anmeldung / Application No. / Demande de brevet n°
PCT/EP 00/00554
Tag des Eingangs / Date of receipt / Date de réception
25/01/00
Zeichen des Anmelders / Vertreters – Applicant / Representative ref. No. – Référence du demandeur ou du mandataire
C 2781 PCT

Anmelder / Applicant / Demandeur :

Datum / Date 26/01/00

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

Das Europäische Patentamt bescheinigt hiermit den Empfang folgender Dokumente :
The European Patent Office hereby acknowledges the receipt of the following :
L'Office européen des brevets accuse réception des documents indiqués ci-dessous :

A. **Internationale** Anmeldung / **International** application / Demande **internationale**

Stückzahl / No. of copies / Nombre d'exemplaires

- [x] Antrag / Request / Requête — 1
- [x] Beschreibung (ohne Sequenzprotokollteil) Description (excluding sequence listing part) Description (sauf partie réservée au listage des séquences) — 3
- [x] Patentansprüche / Claim(s) / Revendication(s) — 3
- [x] Zusammenfassung / Abstract / Abrégé — 3
- [x] Zeichnung(en) / Drawing(s) / Dessin(s) — 3
- [ ] Sequenzprotokollteil der Beschreibung Sequence listing part of description Partie de la description réservée au listage des séquences — —
- [ ] Beigefügte Unterlagen / Accompanying items / Eléments joints — —

B. Beigefügte Dokumente / Accompanying documents / Documents joints

- [ ] Blatt für die Gebührenberechnung Fee calculation sheet Feuille de calcul des taxes
- [ ] Gesonderte unterzeichnete Vollmacht Separate signed power of attorney Pouvoir distinct signé
- [ ] Kopie der allgemeinen Vollmacht Copy of general power of attorney Copie du pouvoir général
- [ ] Prioritätsbeleg(e) Priority document(s) Document(s) de priorité
- [ ] Gesonderte Angaben zu hinterlegten Mikroorganismen oder anderem biologischen Material Separate indications concerning deposited micro-organism or other biological material Indications séparées concernant des micro-organismes ou autre matérial biologique déposés
- [ ] Protokoll der Nucleotid- und/oder Aminosäuresequenzen in computerlesbarer Form Nucleotide and/or amino acid sequence listing in computer readable form Listage des séquences de nucléotides ou d'acides aminés sous forme déchiffrable par ordinateur
- [ ] Abbuchungsauftrag Debit order Ordre de débit — Währung/Currency/Monnaie Betrag/Amount/Montant
- [ ] Scheck Cheque Chèque — *Ausfüllung freigestellt/ Optional/facultatif*
- [ ] Sonstige Unterlagen (einzeln aufführen) Other documents (specify) Autres documents (préciser)

Die genannten Unterlagen sind am oben genannten Tag eingegangen. Die in der Kontrolliste (Feld VIII) des PCT-Antragsformulars RO/101 angegebenen Blattzahlen wurden bei Eingang nicht geprüft. Die Anmeldung hat die ebenfalls oben angeführte Anmeldenummer erhalten / The said items were received on the date indicated above. No check was made on receipt that the number of sheets indicated in the check list (box VIII) of the PCT Request Form RO/101 were correct. The application has been assigned the above-indicated application number / Les documents mentionnés ont été reçus à la date indiquée. L'exactitude du nombre de feuilles indiqué au bordereau (cadre VIII) du formulaire de requête PCT/RO/101 n'a pas été contrôlée lors du dépôt. Le numéro figurant ci-dessus a été attribué à la demande de brevet.

Europäisches Patentamt
European Patent Office
Office européen des brevets
D-80298 München
D. Barbe

Unterschrift / Amtsstempel / Signature / Official Stamp / Signature / Cachet officiel

EPA/EPO/OEB Form 1031.1 01.99

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* C 2781 PCT

Box No. I TITLE OF INVENTION

Immobilization of molecules on surfaces via polymer brushes

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Biochip Technologies GmbH
Engesserstraße 4b
79108 Freiburg im Breisgau
Deutschland

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Klapproth Holger
Kehlerstraße 12
79108 Freiburg
Deutschland

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Vossius & Partner
Siebertstraße 4
81675 München
Deutschland

Telephone No. 089/ 41 30 40

Facsimile No. 089/ 41 30 41 11

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Prucker Oswald
Sertoriusring 283
55126 Mainz
Deutschland

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Rühe Jürgen
Sattlerweg 9a
55128 Mainz
Deutschland

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: DE

State *(that is, country)* of residence: DE

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China
☒ CR Costa Rica
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ DM Dominica
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IN India
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakhstan
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ NO Norway
☒ NZ New Zealand
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia
☒ SK Slovakia
☒ SL Sierra Leone
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ TZ United Republic of Tanzania
☒ UA Ukraine
☒ UG Uganda
☒ US United States of America
☒ UZ Uzbekistan
☒ VN Viet Nam
☒ YU Yugoslavia
☒ ZA South Africa
☒ ZW Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 25.01.1999	99 10 1340.0		EPO	
item (2) 03.03.1999	99 10 4278.9		EPO	
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1) and (2)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date (day/month/year)	Number	Country (or regional Office)
19.08.1999	99104278.9	EPO

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	21
claims	:	3
abstract	:	1
drawings	:	1
sequence listing part of description	:	
Total number of sheets	:	30

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract: Fig. 2

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Renate Barth

Dr. Renate Barth
European Patent Attorney

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: C 2781 PCT

Applicant: Biochip Technologies GmbH
Engesserstraße 4b, 79108 Freiburg i. Br., DE

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . EUR 102.00 [T]

2. SEARCH FEE . . . . . EUR 945.00 [S]

International search to be carried out by EPO

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 30 sheets.

first 30 sheets . . . . . EUR 409.00 [b1]

____ (remaining sheets) x ____ (additional amount) = [b2]

Add amounts entered at b1 and b2 and enter total at B . . . . EUR 409.00 [B]

Designation Fees

The international application contains 83 designations.

8 (number of designation fees payable) x EUR 88.00 (amount of designation fee) = EUR 704.00 [D]

Add amounts entered at B and D and enter total at I . . . . . EUR 1,113.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . EUR 60.00 [P]

5. TOTAL FEES PAYABLE . . . . . EUR 2,220.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

[ ] The designation fees are not paid at this time.

MODE OF PAYMENT

[X] authorization to charge deposit account (see below)	[ ] bank draft	[ ] coupons
[ ] cheque	[ ] cash	[ ] other *(specify)*:
[ ] postal money order	[ ] revenue stamps	

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ EPO

[X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

[X] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

2800.0321	25.02.2000	Dr. Renate Barth
Deposit Account No.	Date *(day/month/year)*	Signature

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EPA

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference: C 2781 PCT
International application No.: PCT/EP00/00554	International filing date *(day/month/year)*: 25 January 2000 (25.01.2000)	(Earliest) Priority date *(day/month/year)*: 25 January 1999 (25.01.1999)

Title of invention:
Immobilization of molecules on surfaces via polymer brushes

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Biochip Technologies GmbH
Engesserstraße 4b
79108 Freiburg im Breisgau
Deutschland

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Klapproth Holger
Kehlerstraße 12
79108 Freiburg
Deutschland

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Prucker Oswald
Sertoriusring 283
55126 Mainz
Deutschland

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

☐ Further applicants are indicated on a continuation sheet.

See Notes to the demand form

Sheet No. 2..

International application No.
PCT/EP00/00554

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code andname of country.)*

Rühe Jürgen
Sattlerweg 9a
55128 Mainz
Deutschland

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code andname of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code andname of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code andname of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/EP00/00554

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

Vossius & Partner
Siebertstr. 4
81675 Munich
DE

Telephone No.: 0049 89 41 30 40

Facsimile No.: 0049 89 41 30 4111

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[x] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

Sheet No. 4

International application No.
PCT/EP00/00554

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [x] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Vossius & Partner
Siebertstr. 4
81675 Munich

(No. 31)

Dr. Renate Barth

European Patent Attorney

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No.	PCT/EP00/00554
Applicant's or agent's file reference	C 2781 PCT

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant

Biochip Technologies GmbH et al.

Calculation of prescribed fees

1. Preliminary examination fee	EUR 1,533.00	P
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	EUR 147.00	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	EUR 1,680.00	TOTAL

Mode of Payment

- [X] authorization to charge deposit account with the IPEA (see below)
- [ ] cash
- [ ] cheque
- [ ] revenue stamps
- [ ] postal money order
- [ ] coupons
- [ ] bank draft
- [ ] other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ EPA [X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

2800.0321 Vossius & Partner	June 27. 2000	Renate Barth
Deposit Account Number	Date *(day/month/year)*	Signature Dr. Renate Barth European Patent Attorney

VOSSIUS & PARTNER

Patentanwälte

Vossius & Partner POB 86 07 67 81634 München Germany

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EUROPEAN TRADEMARK ATTORNEYS
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(bis 1992; danach in anderer Kanzlei)
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Dr. PETER A. RAUH, Dipl.-Chem.
Dr. GERHARD HERMANN, Dipl.-Phys.
JOSEF SCHMIDT, Dipl.-Ing.
Dr. HANS-RAINER JAENICHEN, Dipl.-Biol.
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To the
European Patent Office
Munich

PCT-Patentanmeldung
Nr. PCT/EP00/00554
BIOCHIP TECHNOLOGIES GMBH et al.
"Immobilization of molecules on surfaces via polymer brushes"
u. Z.: C 2781 PCT

11. Januar 2001
Ba/Fi/moe

This is in reply to the Written Opinion dated September 12, 2000:

1. Amendments

As a proposal for further prosecution, an amended claim 1 is submitted which has been reworded as a product by process claim based on process features disclosed in original claim 11.

2. Subject-matter of the present invention

As set out in the introductory portion of the description (cf., e.g., page 2, second and third paragraph) it is a principal object of the present invention to provide a functionalized surface which presents a maximum number of easily accessible coupling sites on a minimum surface area. In order to achieve this goal, the

present inventors have developed a method for the provision of a surface covered with polymer chains (referred to as "polymer brush" in the application) with an increased number of functional sites per unit area and a graft density of the polymer chains which is significantly improved compared to functionalized surfaces known from prior art (cf. page 3, ultimate paragraph bridging to page 4 of the application).

Since the provision of the presently claimed products relies on an entirely new process for their production, the gist of the present invention is best explained in terms of this production method. In order to avoid problems arising from the attempt to position a multitude of polymer chains in close vicinity on a limited surface area, the present invention is based on a two step method. In a first step, polymerization initiators carrying an anchor group are attached to a surface. Since the size of these initiator-anchor units is reduced compared to the final polymer chain and steric hindrance is avoided, a high graft density can be obtained.

Subsequently, polymer chains are grown in situ directly from the surface. The required polymerization process as recited in new claim 1 uses monomers providing functional groups, so that polyfunctional polymer chains are produced which unite to form a polymer brush of high density. Thus, a maximum amount of interaction sites per surface area can be presented to an analyte, e.g. in cases where the modified surface is used as a sensor.

In this context it must be pointed out that the novel process of the invention imparts certain characteristics to the obtained product which help to distinguish the presently claimed polymer brushes from related prior art materials. Therefore, the drafting of a product by process claim to characterize the claimed products appears to be well justified. First of all, functionalized polymers obtained via a polymerization initiator driven reaction differ from the polymeric structures conventionally used to impart a functionality to a given surface, such as polysaccharides which are obtained via polycondensation reactions (cf. figure 2 of D1). Moreover, parts of the initiator molecule, representatives of which are shown on page 11 of the application, will inevitably remain in every single polymer chain of the presently claimed polymer brush, thus marking the product as being obtained according to process features recited in new claim 1. Such structural subunits allow a distinction between the

polymer chain assemblies of the present invention and those disclosed in the cited prior art which are grafted onto the surface in their final form.

In this respect, it should be pointed out that in the light of the present disclosure a wide variety of suitable initiators will be immediately available to the skilled practitioner without requiring more than ordinary skills in the art. As a consequence, it is the applicant's position that the product by process claim 1 in its amended form is appropriate to suitably define the present invention and to delimit it from the prior art.

3. Novelty

3.1 Regarding D1

3.1.1 As opposed to the polymer brush of the present invention, the biosensor device of WO 97/41425 (D1) is not comprised of polymer chains having a multitude of functional groups. Rather, as explained e.g. on page 3, line 30 or page 4, lines 26 and 27 of D1, this prior art document discloses an assembly of polymer chains which are monofunctional, i.e. which carry a single ligand per polymer chain which is suitable for a coupling reaction with an analyte.

Even if a combination of hydrocarbon chains and ligands, illustrated e.g. in figure 2 of D1, should be compared to the final polymer brush of the present invention, these prior art embodiments were not embraced by the definition of present claim 1. For once, such polysaccharide structures are produced via polycondensation reactions and are not obtainable by a polymer initiator driven polymerization reaction as stipulated in present claim 1. Moreover, as set out on page 7, line 2 and lines 28-31 of D1, the chain structures of this figure are formed by hydrocarbon chains anchored to the surface (26), which are combined with a polysaccharide (52). Therefore, the definition given in D1 for this figure 2 leaves no room for structural subunits in the polymer chains resulting from the presence of a polymerisation initiator.

3.1.2 The processes disclosed by D1 for the provision of a polymer chain assembly from page 8, line 25 to page 11, line 9 differ completely from the process of present claim 11. In all cases, D1 uses complete hydrocarbon chains to which an anchor molecule, such as a thiol coupling group, is attached and which are

either directed towards the surface via passive diffusion (figure 3A) or by means of an electric field (figure 3B and C). Thus, the prior art document is far from disclosing an in situ polymerization method carried out directly on the final surface to provide a polyfunctional polymer monolayer as it is claimed in present claim 11.

3.2 Regarding D2

3.2.1 With regard to polymer structures of WO 90/05303 (D2), the same arguments apply as with regard to those of D1. Thus, it should be understood from the definition of the structural formula X-R-Y on pages 6 and 7 of D2 that this invention does not aim at the provision of multifunctional polymer chains which allow an interaction of the polymer with a multitude of sample or probe molecules as defined in present claim 1. Rather, D1 defines one target-binding group, Y or Y' respectively, to be present per polymer chain R or R'. Just as in the case of D1, the definition of the polymer chains R and R' in D2 does not allow for units derived from a polymerization initiator to be present, and it can be seen from the examples that for the purpose of D2 R or R' are preferred to be simple hydrocarbon chains which do not interact with target molecules. Thus, the structure of the single units X-R-Y of the polymer chain assemblies of D2 differs significantly from the polyfunctional polymer chains of the present invention.

3.2.2 Contrary to the process of the present application, the functionalized polymers of D2 are integrally attached to the sensor substrate (confirm page 9, ultimate paragraph of D2) in a single step. Again, an anticipation of the process of present claim 11 must be denied.

3.3 Regarding D3

C.P. Sönksen et al., Anal. Chem., 70, 1998, 2731-2736 (D3) is related to the interaction of myoglobin with a monoclonal IgG functionalized gold surface and to the interaction of a biotinylated DNA with streptavidin functionalized surfaces. However, no information can be obtained from this document regarding the exact nature of the polymers carrying the biospecific ligands or the process for

their production. Accordingly, novelty of the present invention as explained under item 2 above should not be drawn into question in view of this document.

4. Inventive step

4.1 The problems involved with conventional monolayers (as disclosed in D1 to D3) on a biosensor surface, e.g. limited and not well defined graft density, low density of available interaction sites etc., are discussed in detail in the introduction of the present invention, see particularly page 2, paragraph 3.

4.2 The invention is based on the surprising finding that with polyfunctional polymers as defined in present claim 1 these problems may be easily overcome. None of the cited references, neither taken alone nor in combination, gives the slightest hint or clue, let alone any specific guidance, to the skilled artisan which would motivate him to open up new ways, i.e. to use polyfunctional polymers prepared as defined in present claim 1 instead of the conventional bifunctional polymers described in D1 to D3. Thus, the cited prior art cannot render the claimed invention obvious.

4.3 The advantages of the inventive approach are impressing. The number of molecules interacting per surface unit, i.e. the graft density, and the density of available interaction sites are markedly increased. The graft density and the chain length, and thus the thickness, of the polymer layer may be easily controlled by choosing the appropriate polymerization conditions. It is also possible to fine-tune the properties of the resulting layer, e.g. with respect to the access of the functional groups for subsequently coupled sample or probe molecules which may vary considerably in their size and structure. Since the flexibility of the polymer chains allows a complete coverage of a sensor surface, surface effects, e.g. during laser scanning, can be avoided.

4.4 In addition, it should be noted that since the polymer layers of the invention are prepared directly on a surface, even surfaces which are inaccessible for conventional modification methods may be provided with the polymer monolayers because no bulky polymers have to diffuse towards the surface. Moreover, structured surfaces can be provided, e.g. by starting the polymerization from patterned arrays of initiator molecules.

4.5 In view of the fundamental differences between the disclosure content of the present invention and the teaching of the cited prior art documents discussed above, it is the applicant's position that the inventive concept of the present invention as explained under item 2 can not be affected by any of D1 to D3 alone or in combination. The presence of an inventive step should thus be acknowledged.

5. Requests

With the above explanations and the proposed modifications to the claims, we feel that Applicant has met the requirements of the PCT. It is respectfully requested that the objections of the Examiner be withdrawn and that a favorable IPER be issued. If the Examiner requires additional information or explanation, applicant requests that he be given an opportunity to provide such information or explanation prior to the issuance of the IPER. Minor questions may also be discussed via the phone.

Renate Barth

Dr. Renate Barth
European Patent Attorney

Enclosure
Claims set including amended claim 1

Claims

1. Polyfunctional polymer monolayer comprising an assembly of polymer chains attached to a surface obtainable by a process comprising the steps of:

    a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

    b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules,

    wherein each polymer chain comprises a multitude of identical or different units carrying one or more functional groups which allow an interaction of the polymer with a sample or probe molecule.

2. Polymer monolayer according to claim 1, wherein the polymer chains are covalently attached to the surface.

3. Polymer monolayer according to claim 1 or 2, wherein the functional groups are chosen from carboxylic acids, maleinimides, N-hydroxy succinimides, epoxides, isothiocyanates, isocyanates or azides.

4. Polymer monolayer according to any of claims 1 to 3, wherein the sample molecule or probe molecule is chosen from proteins, peptides, polysaccharides or nucleic acids and derivatives thereof.

5. Polymer monolayer according to any of claims 1 to 4, wherein the polymer comprises segments that make the layer water swellable.

6. Polymer monolayer according to claim 5, wherein the water swellability is provided by monomers chosen from acrylic acid, methacrylic acid, dimethyl acrylamide or vinyl pyrrolidon.

7. Polymer monolayer according to any of claims 1 to 3, 5 and 6, further comprising a multitude of identical or different probe molecules immobilized at the polymer chain via a reaction with the functional groups.

8. Polymer monolayer according to claim 7, wherein the probe molecules are selected from nucleic acids, PNAs, polysaccharides, proteins and peptides.

9. Surface carrying a polyfunctional polymer monolayer according to any of claims 1 to 8.

10. Surface according to claim 9, wherein the polymer chains are in the form of patterned arrays.

11. Process for the production of a polyfunctional polymer monolayer according to any of claims 1 to 8, comprising the steps of:

    a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

    b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules.

12. Process according to claim 11, wherein the initiator comprises a chlorosilane, an alkoxysilane, a disulphide or a thiol group.

13. Process according to claims 11 or 12 wherein the initiator comprises a group chosen from azo groups, peroxo groups, or a ketone group in conjugation with an aromatic system.

14. Process according to claim 13, wherein the initiator comprises a group chosen from aromatic ketones or aromatic ketones containing sulphur.

15. Process for the detection of sample nucleic acid molecules, using a polymer monolayer according to claim 7 or 8, which comprises the steps of

    a) allowing a hybridization reaction to take place between the probe and the sample, followed by
    b) removal of the non hybridized nucleic acid molecules in a washing step and
    c) detection of the hybridized nucleic acid molecules.

16. A process for purifying a compound from a sample comprising the steps of

    (a) contacting the sample with the polymer monolayer of any of claims 1 to 8, under conditions that allow binding of said compound to the functional group of the polymer chain or the probe molecule;
    (b) and removing material from the sample that has not bound to the polymer layer or a probe molecule;

17. The process according to claim 16 further comprising

    (c) eluting the bound complex from the polymer layer.

18. The process according to claim 16 or 17, wherein said compound is a nucleic acid, a (poly)saccharide or a (poly)peptide or a complex thereof, preferably an antibody or a fragment or derivative thereof.

19. Use of the surface according to claims 9 or 10 as an affinity matrix.

20. Use of a surface according to claims 9 or 10 in a sensor chip.

21. Medical or diagnostic instrument, comprising a surface according to claims 9 or 10.

22. Use of a surface according to claims 9 or 10 for the immobilization of starter molecules for the formation of oligo- or polymers, preferably for nucleic acid or peptide synthesis.

23. Use of polymer layer according to any of claims 1 to 8 as a gel in the separation of molecules in an electric field.

14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 2781 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/00554	25/01/2000	25/01/1999
International Patent Classification (IPC) or national classification and IPC		
C12Q1/68		
Applicant		
BIOCHIP TECHNOLOGIES GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/06/2000	12.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moreno de Vega, C Telephone No. +49 89 2399 7486

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/00554

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-21	as originally filed				
Claims, No.:					
1-23	as received on	12/01/2001	with letter of	11/01/2001	
Drawings, sheets:					
1/1	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6, 10-14
	No:	Claims	1-5, 7-9, 15-23
Inventive step (IS)	Yes:	Claims	11-14
	No:	Claims	1-10, 15-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 97 41425 A (PENCE INC ;UNIV MCGILL (CA)) 6 November 1997 (1997-11-06)

D2: WO 90 05303 A (PHARMACIA AB) 17 May 1990 (1990-05-17)

D3: SÖNKSEN C.P. ET AL.: 'Combining Maldi mass spectrometry and biomolecular interaction analysis using a biomolecular interaction analysis instrument' ANAL. CHEM., vol. 70, - 1 July 1998 (1998-07-01) pages 2731-2736

The International Examining Authority in charge of this application has considered the applicant's arguments in response to the Written Opinion, nevertheless the following objections are still maintained. Furthermore, the feature "obtainable by the process comprising the steps of...a)... b)..." in claim 1 is not limiting the scope of the claim (see further Item VIII).

1. Novelty (Article 33 (2) PCT)

1.1 Claims 1-5, 7-9, 15-23 are not considered to be novel, as they are already disclosed in the prior art.

D1 (see page 4 lines 5-18, example 2, claims and figures) discloses a biosensor apparatus for detecting a binding event between a ligand and a receptor. The biosensor includes an electrode substrate coated with a high-dielectric hydrocarbon-chain monolayer, having ligands attached to the exposed monolayer surface and measuring the binding of a receptor to the monolayer-bound ligand. The hydrocarbon chains are attached via a thiol group. This document appears to be novelty destroying for claims 1-5, 7-9, 15-23.

D2 (see especially pages 6-8, claims) discloses methods for the production, on metal surfaces, of surface layers which are capable of selective biomolecular interactions, sensing surfaces produced by means of these methods and the use thereof in biosensors. The surface layers are

monolayers of organic molecules (e.g. hydrocarbon chains) attached by thiol, nitro, etc. groups and which contains active groups that bind ligands and biomolecules. This document appears to be novelty destroying for claims 1-5, 7-9, 19-21.
D3 (see especially page 2732) studies the interaction of binding of myoglobin to an immobilized (on a carboxymethyl-dextran/gold surface of a sensor chip) monoclonal IgG directed against human myoglobin and capturing of the DNA binding protein PaR by a biotinylated double-stranded (ds) DNA probe bound to the sensor chip via biotin-streptavidin interaction. This document appears to be novelty destroying for claims 1, 9, 16-21.

1.2 Claims 6, 10-14 are considered to be novel, as their subject-matter is not disclosed in the prior art.

2. Inventive step (Article 33(3) PCT)
Claims 6 and 10 differ from D1, which is considered to be the most relevant prior art, in that the polymer contains specific monomers which provide water swellability and in that the polymer chains are disposed on the surface in patterned arrays. The technical problem to be solved by these claims is the provision of improved surfaces useful as biosensors. D1 and D2 solve the same technical problem; claims 6 and 10 do not contain any feature which in combination with the features of the claims to which they refer, in the light of D1, D2 and the general knowledge in the field, meet the requirements of the PCT with respect to inventive step. Thus, claims 6 and 10 are not considered to be inventive.

Claims 11 to 14 differ from D2, considered to be the most relevant prior art for these claims, in the method of producing the polyfunctional polymer monolayer, i.e. said method comprises first covering the surface with a monolayer of polymerization initiator with groups suitable to attach it to the surface, and carrying out a polymerization in the presence of monomers with functional groups which allow the coupling. If the polymer obtained with the specific molecules. The technical problem to be solved by these claims is the provision of a method of producing improved polyfunctional polymer monolayers useful in the detection of biological molecules. There is not hint

in the prior art to arrive at the solution proposed by these claims. Thus, claims 11-14 are considered to be inventive.

Re Item VII
Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2 and D3 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

Claim 1 is not clear (Article 6 PCT), as it intends to define its subject-matter (product) in terms of the process used to obtain the product claimed.

Claims

1. Polyfunctional polymer monolayer comprising an assembly of polymer chains attached to a surface, characterized in that each polymer chain comprises a multitude of identical or different units carrying one or more functional groups which allow an interaction of the polymer with a sample or probe molecule.

2. Polymer monolayer according to claim 1, wherein the polymer chains are covalently attached to the surface.

3. Polymer monolayer according to claim 1 or 2, wherein the functional groups are chosen from carboxylic acids, maleinimides, N-hydroxy succinimides, epoxides, isothiocyanates, isocyanates or azides.

4. Polymer monolayer according to any of claims 1 to 3, wherein the sample molecule or probe molecule is chosen from proteins, peptides, polysaccharides or nucleic acids and derivatives thereof.

5. Polymer monolayer according to any of claims 1 to 4, wherein the polymer comprises segments that make the layer water swellable.

6. Polymer monolayer according to claim 5, wherein the water swellability is provided by monomers chosen from acrylic acid, methacrylic acid, dimethyl acrylamide or vinyl pyrrolidon.

7. Polymer monolayer according to any of claims 1 to 3, 5 and 6, further comprising a multitude of identical or different probe molecules immobilized at the polymer chain via a reaction with the functional groups.

8. Polymer monolayer according to claim 7, wherein the probe molecules are selected from nucleic acids, PNAs, polysaccharides, proteins and peptides.

9. Surface carrying a polyfunctional polymer monolayer according to any of claims 1 to 8.

10. Surface according to claim 9, wherein the polymer chains are in the form of patterned arrays.

11. Process for the production of a polyfunctional polymer monolayer according to any of claims 1 to 8, comprising the steps of:

    a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

    b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules.

12. Process according to claim 11, wherein the initiator comprises a chlorosilane, an alkoxysilane, a disulphide or a thiol group.

13. Process according to claims 11 or 12 wherein the initiator comprises a group chosen from azo groups, peroxo groups, or a ketone group in conjugation with an aromatic system.

14. Process according to claim 13, wherein the initiator comprises a group chosen from aromatic ketones or aromatic ketones containing sulphur.

15. Process for the detection of sample nucleic acid molecules, using a polymer monolayer according to claim 7 or 8, which comprises the steps of

    a) allowing a hybridization reaction to take place between the probe and the sample, followed by
    b) removal of the non hybridized nucleic acid molecules in a washing step and
    c) detection of the hybridized nucleic acid molecules.

16. A process for purifying a compound from a sample comprising the steps of

    (a) contacting the sample with the polymer monolayer of any of claims 1 to 8, under conditions that allow binding of said compound to the functional group of the polymer chain or the probe molecule;
    (b) and removing material from the sample that has not bound to the polymer layer or a probe molecule;

17. The process according to claim 16 further comprising

    (c) eluting the bound complex from the polymer layer.

18. The process according to claim 16 or 17, wherein said compound is a nucleic acid, a (poly)saccharide or a (poly)peptide or a complex thereof, preferably an antibody or a fragment or derivative thereof.

19. Use of the surface according to claims 9 or 10 as an affinity matrix.

20. Use of a surface according to claims 9 or 10 in a sensor chip.

21. Medical or diagnostic instrument, comprising a surface according to claims 9 or 10.

22. Use of a surface according to claims 9 or 10 for the immobilization of starter molecules for the formation of oligo- or polymers, preferably for nucleic acid or peptide synthesis.

23. Use of polymer layer according to any of claims 1 to 8 as a gel in the separation of molecules in an electric field.

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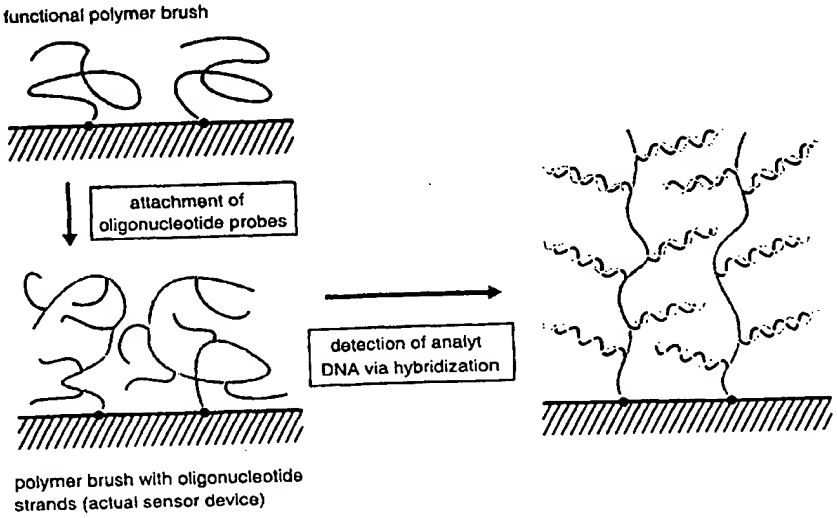
(54) Title: IMMOBILIZATION OF MOLECULES ON SURFACES VIA POLYMER BRUSHES



(57) Abstract

The invention relates to polyfunctional polymer monolayers (polymer brushes) comprising a multitude of polymer chains attached to a surface, with each polymer chain comprising a multitude of units carrying at least on functional group which allows the interaction of the polymer chain with a sample molecule.

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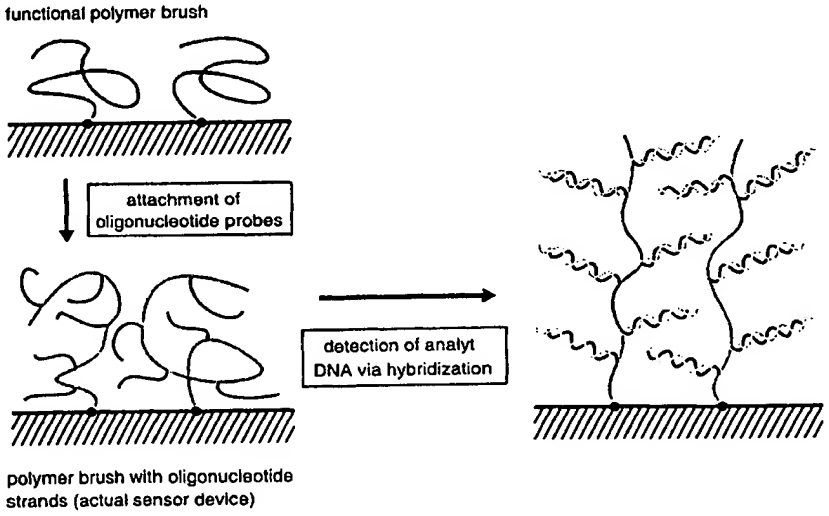
(54) Title: IMMOBILIZATION OF MOLECULES ON SURFACES VIA POLYMER BRUSHES



(57) Abstract

The invention relates to polyfunctional polymer monolayers (polymer brushes) comprising a multitude of polymer chains attached to a surface, with each polymer chain comprising a multitude of units carrying at least on functional group which allows the interaction of the polymer chain with a sample molecule.

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NZ	New Zealand
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PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Immobilization of molecules on surfaces via polymer brushes

Due to the steadily growing importance of microtechniques in a wide variety of scientific applications, the development of systems which allow the interaction of molecules with surfaces remains a critical issue. Such interactions include the possibility of removing specific molecules from a sample, e.g. to facilitate their analysis/detection, but also of presenting molecules on a surface, thus allowing subsequent reactions to take place. These principles for the immobilization of molecules can be applied in sensor or chromatographic systems or for the provision of modified surfaces in general.

In recent years there have been numerous approaches to fabricate sensor chips which are based on self-assembled monolayers (SAM's) of bifunctional molecules which directly or indirectly couple sample molecules to the sensor surface. Typically, these bifunctional molecules carry a silane or thiol/disulfide moiety in order to achieve a bond with the inorganic surface and an additional functional group (e.g. amino or epoxide groups) which interact with sample molecules, often contained in biological samples in the form of an oligonucleotide, a protein or a polysaccharide etc.

While the formation of a direct bond between the bifunctional compound and the sample molecule is possible, the sample molecules do not necessarily interact directly with the couplers forming the monolayer. Alternatively, appropriate immobilized biomolecules themselves can act as probes for the detection of sample molecules. Such probe molecules can equally be immobilized via a reaction with the free functional groups of the monolayer. In particular, if biomolecules are used as probe molecules, their presence may significantly enhance the specificity of the interaction of the sample molecules with the modified surface. For example, in cases where the fast analysis of a sample of DNA fragments or molecules is required, the monolayers of bifunctional molecules can first be brought in contact with synthetic oligonucleotides which will thus be

immobilized. Subsequently, the hybridization of specific molecules, such as compatible strands from a sample is detected, e.g. via fluorescence microscopy, if dye-labeled sample molecules are used.

Figure 1 shows a schematic description (not to scale) of the design of conventional DNA sensors. Monolayers of usually synthetic oligonucleotide single strands as probes are immobilized on a surface and serve as probe molecules for complementary sample oligonucleotides which are bound via hybridization. The hybridization reaction is, for example, detected via fluorescence originating from appropriate dye labels that are attached to the sample molecules.

Although these techniques are well established for this purpose, the application of standard detection methods is problematic, especially in cases where the surface area available for the detection of one specific type of sample molecules is restricted, e.g. if a variety of molecules is to be analyzed in a parallel process, since the monolayers are limited in their graft density. For example, since the number of hybridized double strands per surface unit of a sensor can not easily be increased, suitable detectors have to meet very high requirements with regard to their sensitivity. Thus, the minimum surface area on a sensor necessary for the detection of one type of oligonucleotide can not be easily reduced. Moreover, the maximum density, i.e. one sample or probe molecule per functional group of the couplers can hardly be attained, since due to sterical hindrance on the two-dimensionally extended monolayer, only a fraction of the functional groups will be able to react with sample or probe molecules. Thus, the overall graft density is low and normally not well defined.

Similar problems with regard to the limited number of reaction sites per surface unit can arise in other applications, where it is desirable to immobilize an increased amount of molecules on a surface.

Various attempts have been made to overcome the problems outlined above. As regards the analysis of oligonucleotides, it has been tried to increase the graft density on the surface by using oligomers or polymers which carry an oligonucleotide strand (or a functional group for its attachment) together with a suitable group which allows the bonding of these oligomers or polymers to the surface of the sensor chip. Due to the increased flexibility of the oligomeric or polymeric chains, a larger fraction of the bifunctional oligomer or polymer

molecules which are coupled to the surface is able to immobilize oligonucleotide probe molecules.

However, the total oligonucleotide graft density is not significantly increased, because the graft density of the bifunctional oligomeric or polymeric molecules on the surface is limited. This is a consequence of the fact that the self-assembly of the oligomers or polymers is hindered for kinetic reasons, because once the sensor surface is covered with such molecules, further polymers will have to diffuse against a concentration gradient in order to reach the surface.

Accordingly, it is an object of the present invention to provide a surface which is modified with a polymer monolayer comprising functional groups for the interaction with sample or probe molecules, wherein the number of molecules interacting per surface unit is markedly increased compared to conventional (short chain) monolayers of bifunctional molecules. In addition, the density of available interaction sites should be higher than that obtained from the reaction of bifunctional polymers or oligomers with the surface.

In the specific case of the detection of DNA molecules such as oligonucleotides, the object can be expressed as the provision of a surface with a graft density of synthetic oligonucleotide strands which is higher than that created by coupling the respective oligonucleotides to a functionalized monolayer of low molecular weight couplers. Also, the graft density should be higher then that resulting from the reaction of polymers or oligomers modified with a synthetic oligonucleotide single strand with the surface.

This object has been achieved by a surface to which an assembly of polymer chains is attached, which comprise each a multitude of functional groups that allow an interaction of the polymer with sample or probe molecules. If, for example, such a polyfunctional polymer chain is used to immobilize one or more synthetic oligonucleotide probes, complementary nucleic acids can subsequently be detected from a mixture of sample molecules after a hybridization reaction has taken place. Surprisingly, it has been found that such an assembly of polyfunctional polymer chains, also referred to as a polymer brush, does not suffer from the problems of conventional detection methods where a high graft density could not be achieved. Moreover, since the flexibility of the polymer chains allows

a complete coverage of the sensor surface, surface effects, e.g. during laser scanning, can be avoided.

The term „interaction", as used in this specification includes the formation of covalent bonds, as well as attractive ionic and van-der-Waal's forces and hydrogen bonds. The respective functional moiety within the polymer chain or the probe molecules, which defines the type of interaction, will be selected according to the desired application of the surface according to the invention.

The expression „immobilize" is used hereinafter for an interaction of molecules with the polymer brushes resulting in the formation of a bond which is permanent under the chosen conditions. For example, probe molecules are immobilized by the polymer brushes during their application on a sensor surface. However, by changing conditions (e.g. pH-value, ionic strength) an immobilization may sometimes be reversed.

The term „sample molecule" shall be used herein for molecules which are present in a sample and which couple temporarily or permanently to the polymer chains according to the invention. The present invention includes two general principles for an interaction of the claimed polymer brushes with the sample molecules. In a first embodiment, the functional groups comprised within the polymer chains are chosen in order to allow a direct interaction of the chains with the sample molecules. In a second embodiment, probe molecules are immobilized at the functional groups of the polymer brush, and an interaction takes place between those probe molecules and the sample molecules.

Suitable probe molecules are molecules which are at least bifunctional, so that after their coupling to the multifunctional polymer chains new interaction sites are present in the polymer monolayer according to the invention, which allow an interaction with sample molecules. Preferably, the probe molecules provide highly specific interaction sites for the sample molecules. They can be derived from natural or non-natural sources. Particularly preferred probe molecules are biomolecules such as nucleic acids, including DNA, RNA or PNA (peptide nucleic acid), most preferably oligonucleotides or aptamers, polysaccharides, proteins including glycosidically modified proteins or antibodies, enzymes, cytokines, chemokines, peptidhormones or antibiotics, and peptides. In order to ensure a

sufficient stability, e.g. during a sensor application, the probe molecules are preferably covalently bound to the polymer brush.

Depending on use, a multitude of identical probe molecules or a mixture of two or more different probes may be immobilized. For example, a set of identical probe molecules is preferred for the application of the polymer brushes as an affinity matrix.

The polymer monolayer according to the present invention comprises a multitude of single polymer chains which are attached to a surface. Preferably the bond between the polymer chains and the surface is covalent. It is also preferred that the polymer chains are attached to the surface at one of their terminals. The introduction of branched polymers is possible, if desired.

Figure 2 shows a schematic illustration (not to scale) of the design of DNA sensors based on functional polymer brushes. The single stranded oligonucleotides that serve as probe molecules are attached to surface anchored polymer chains. Sample oligonucleotides are detected via hybridization. This reaction can be detected by measuring the significant increase in the layer thickness caused by the incorporation of additional material into the layer.

The polymer chains of the present invention may be homo- or copolymers, depending on the desired application. A homopolymer would be represented by a polymer wherein each of the monomeric units used in polymerization carries at least one of the functional groups which can interact with sample or probe molecules. However, in order to impart certain advantageous properties to the polymer monolayer, a copolymer, formed from these monomers with specific functional groups for the interaction with sample or probe molecules (hereinafter referred to as „functionalized monomers") together with other comonomers can be used.

For example, the reaction of the sample or probe molecules with the polymer is significantly facilitated if the polymer is swellable in the solvent containing these molecules, so that comonomers should preferably be chosen which show a strong interaction with the solvent in question. Since, in a most preferred embodiment of the present invention, biomolecules, which are normally present in aqueous

solutions, interact with the polymer chains, said polymer chains are preferably water swellable.

Thus, for example, one or more comonomers can be used which are polar, or even soluble in water, if a homopolymer of functionalized monomers does not show sufficient interaction with water to allow a fast reaction of the molecules to be detected with the functional groups. Both types of monomer, functionalized as well as comonomers, preferably contain a C-C double bond which can react in a radical polymerization reaction. Examples for suitable comonomers which yield a water swellable polymer are acrylic acid, methacrylic acid and derivatives thereof, as e.g. esters and amides of these acids, with alcohols or amines preferably comprising 1 to 12 carbon atoms.

Common examples of this group of monomers are hydroxyethyl methacrylate, acrylamide and dimethyl acrylamide. Another suitable monomer is vinyl pyrrolidon. It is also possible to use monomers that yield at first water insoluble polymers which can then be transferred to water soluble derivatives. A suitable example for this group of polymers is polyvinyl alcohol which can be obtained, for example, by saponification of polyvinyl acetate.

If a copolymer is used, the ratio of comonomers to functionalized monomers is determined prior to the polymerization process in order to define the composition of the resulting polymer chain. Preferably, the ratio of the comonomers to the functionalized monomers ranges from 50/1 to 1/1, more preferably form 20/1 to 2/1.

The functional groups which are necessary to allow a interaction of the polymer layer with the sample or probe molecules are preferably present in side chains of the polymer chains. A „multitude" of functional groups comprised in the polymer chains of the monolayer of the present invention means at least two, but preferably more than two groups per polymer chain. Since the concerned functional groups are preferably comprised in monomers forming the polymer brushes, their number may amount up to several thousand, e.g. up to 10000 of these groups present in a single chain, depending on the size of the probe or sample molecule to be immobilized. Preferably, each chain comprises 20 to 1000 of these functional groups.

Suitable functionalized monomers which are present in the polymer brushes are those monomers which comprise a polymerizable C-C double bond, as well as a further functional moiety that does not take part in the polymerization process. Preferably, this functional group is linked to the main polymer chain via a C_2-C_{10}, more preferably a C_3-C_7 alkyl chain as a spacer.
The spacer molecules can be part of the functionalized monomers. Suitable monomers for this approach include acrylic and methacrylic esters or amides of C_2-C_{10} alcohols or C_2-C_{10} amines. In order to serve as spacers, these alcohols or amines carry an additional functional group at the terminal opposite to the one forming the ester or amide bond. This functional group either represents the one necessary for the interaction with the sample or probe molecules, or can be transformed to such a suitable functional group in a further step.

Alternatively, it is also possible to attach these spacer molecules to suitable reactive segments within the polymer monolayer after its formation. In this case, reactive monomers have to be present during polymerization, such as acrylic or methacrylic acid chlorides or reactive esters thereof, as N-hydroxy succinimides or other monomers, e.g. maleic anhydride. These preferred reactive monomers can form covalent bonds to the bifunctional alcohols or amines that may be used as spacers.

The monomers carrying the spacer unit can readily be synthesized from the respective acrylic or methacrylic acid chloride or anhydride and the ω-amino or hydroxy carboxylic acid. The resulting product can be transformed to the active ester derivative by using e.g. N-hydroxy succinimide. A detailed procedure for the synthesis of several examples of such monomers can be found in the literature e.g., in H.-G. Batz, J. Koldehoff, Macromol. Chem. 177 (1976)683.

As outlined above, it is possible to use reactive monomers which directly yield a polyfunctional polymer monolayer according to the invention. Alternatively, monomers can be chosen which carry a precursor of the functional group to be used on the final surface, e.g. an acid chloride or an acid anhydride. They can subsequently be transformed to reactive groups, e.g. NHS ester or glycidylester groups, which allow an interaction of the polymer with sample or probe molecules under the desired conditions.

Thus, all polymerizable monomers are suitable for the purposes of the present invention, as long as they can be combined with, or comprise, functional groups necessary to allow an interaction of the polymer with the sample molecules or probe molecules.

Functional groups which can be used for the purposes of the present invention are preferably chosen according to the molecules with which an interaction is to be achieved. The interaction can be directed to one single type of sample molecule, or to a variety of sample molecules. Since one important application of the present invention is the detection of specific molecules in biological samples, the functional groups present within the polymer brushes will preferably interact with natural or synthetic biomolecules which are capable of specifically interacting with the molecules in biological samples, leading to their detection. Suitable functional moieties will preferably be able to react with nucleic acids and derivatives thereof; such as DNA, RNA or PNA, e.g. oligonucleotides or aptamers, polysaccharides, proteins including glycosidically modified proteins or antibodies, enzymes, cytokines, chemokines, peptidhormones or antibiotics or peptides or labeled derivatives thereof.

Moreover, it will be possible to conduct the coupling reaction between the molecules to be detected or the synthetic oligonucleotides and the polymer chains under conditions which are not detrimental to the sample or probe molecules. Consequently, in an nucleic acid sensor application, the reaction should be carried out in an aqueous solution, and the temperature should not be raised above 95°C.

Also, the coupling reaction should proceed at a reasonable rate so that the detection can preferably be accomplished within less than 24 hours without requiring extreme pH-values in the solution. For the immobilization of synthetic oligonucleotide single strands, the pH should range between 7 and 11, preferably 7 to 10. During the hybridization reaction of the nucleic acid sample molecules with the probe molecules, the bond between the functional group and the synthetic oligonucleotide single strand as well as the bond of the polymer chain to the substrate has to be able to withstand temperatures of more than 65 °C, and a pH of 6-9. In cases where DNA is used as a sample molecule, the temperatures may have to be raised up to about 95°C in order to effect a separation of the DNA strands, which is necessary for hybridization.

Since most of the probe molecules, especially in biological or medical applications, comprise sterically unhindered nucleophilic moieties, preferred interactions with the polymer brushes comprise nucleophilic substitution or addition reactions leading to a covalent bond between the polymer chains and the sample or probe molecules. For example, synthetical oligonucleotides are usually provided with a free amine group at one end (5´or 3´). Thus, exemplary functional groups provide, for example, a reactive double bond, an equivalent for a double bond (as e.g. an epoxy group) or a reactive leaving group. However, ionic or van-der-Waals forces as well as hydrogen bonds can also be used to couple sample molecules to the polymer brushes if their functional groups are chosen accordingly.
With appropriate functional groups present in the polymer brushes, the polymer monolayers of the present invention can also be used in separation methods, e.g. as a stationary phase in chromatographic applications.

Preferred functional groups can be chosen from prior art literature with respect to the classes of molecules which are to be immobilized and according to the other requirements (reaction time, temperature, pH value) as described above. A general list can for example be found in the text book „Bioconjugate Techniques" by G. T. Hermanson, Academic Press, 1996. In the case of the attachment of amino-terminated oligonucleotides, examples for suitable groups are so-called active or reactive esters as N-hydroxy succinimides (NHS-esters), epoxides, preferably glycidyl derivatives, isothiocyanates, isocyanates, azides, carboxylic acid groups or maleinimides.

As preferred functional monomers which directly result in a polyfuncional polymer monolayer, the following compounds can be employed for the purposes of the present invention:

- acrylic or methacrylic acid N-hydroxysuccinimides,
- N-methacryloyl-6-aminopropanoic acid hydroxysuccinimide ester,
- N-methacryloyl-6-aminocapronic acid hydroxysuccinimide ester or
- acrylic or methacryl acid glycidyl esters.

Depending on the application, there is the possibility of providing a polymer brush with a combination of two or more different functional groups, e.g. by carrying out

the polymerization leading to the polymer chains in the presence of different types of functionalized monomers. Alternatively, the funcional groups may be identical.

The preferred method for the preparation of the polyfunctional polymer monolayer according to the invention is described in the following:

In a first step, the surface is covered by a monolayer of polymerization initiators or starter molecules. The groups in these initiators which allow the initiation of the polymerization are usually chosen e.g. from peroxo groups or azo groups if a thermally initiated radical mechanism is to be used. Aromatic ketones such as benzoin, benzil or benzophenone derivatives are preferably used if the polymers are formed by photochemical initiation. Aromatic ketones comprising sulphur may equally be used, if desired, in order to shift the suitable wavelength for photoinitation to a longer wavelength region. In addition to such labile groups, suitable initiators for the preferred process according to the invention carry one or more groups suitable for their attachment to the surface to be covered by the polymer chains.

The polymer chains according to the present invention are usually grown from the surface via a chain reaction. While radical mechanisms are preferred for practical reasons, the application of ionic polymerization techniques is also possible.

The functional groups comprised in the initiator molecules for surface attachment have to be adapted to the sensor surface used. For the preparation of the initiator monolayer on metal oxides, especially silicon oxide surfaces (evaporated or sputtered SiO_x layers, SiO_2 surfaces of silicon wafers, glass, quartz), chlorosilane moieties or alkoxysilanes are used. Thiol or disulfide groups can be employed for the modification of gold surfaces. However, silanes are usually preferred due to their increased stability on surfaces. Moreover, the present invention is not restricted to inorganic surfaces. Organic polymer surfaces can also be used as substrates to carry the polymer monolayers, and there is also the possibility to include the starters for the polymerization reaction directly into such a surface forming polymer.

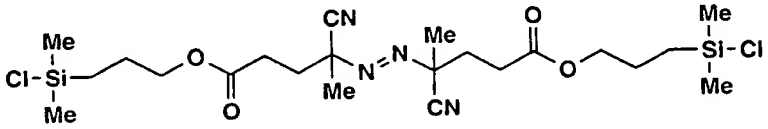
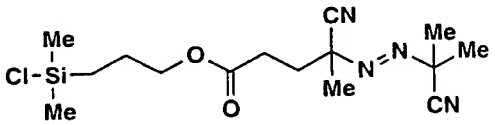
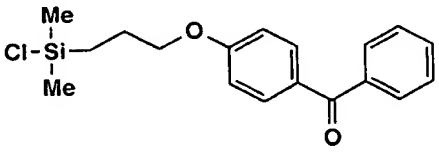
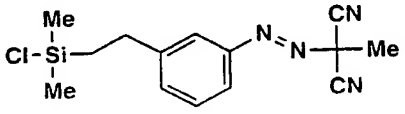
Preferred examples for initiators which can be used for the purposes of the present invention are listed below, together with their structure formulae:

-4,4´-Azobis-(4-cyano pentanoic acid (3´-chlorodimethylsilyl) propyl ester), compound 1 or the respective di- and trichloro or mono-, di- and trialkoxy silane analogs;

-2,4´-Azo-(4-cyano pentanoic acid (3´´-chlorodimethylsilyl) propyl ester), compound 2 or the respective di- and trichloro or mono-, di- and trialkoxy silane analogs; or the respective compounds with an undecyl spacer rather than an propyl spacer; or disulfide or thiol derivatives of this general type of azo compounds;

-4-(3´-chlorodimethylsilyl)propyloxy) benzophenone, 3 or the respective di- and trichloro- or mono-, di- and trialkoxy silane analogs;

-silane and disulfide/thiol derivatives of arylazomalodinitriles, such as compound 4.

compound number	structure
1	
2	
3	
4	

Upon initiation of the polymerization reaction, preferably by a heating step (thermal initiation) or exposure to radiation (photoinitiation) in the presence of polymerizable monomers, polymer chains can be grown from the surface. The polymerization can be carried out under standard reaction conditions known in the art. If this technique is applied, the graft densities of the resulting polymer monolayer can be controlled over a wide range, for example by variation of the polymerization time. Moreover, graft densities can be achieved that are inaccessible by other methods. Thus, polymers can be attached such that the average distance between to anchoring sites on the surface is 5 nm or less, e.g. 2 to 5 nm. Advantageously, such graft densities can be achieved independent of the molecular weight of the attached chains, e.g. even for molecular weights of 100000 g/mol or more.

Furthermore, the preferred in-situ formation of polymer chains on a surface according to the present invention allows the control of the average molecular weight of the attached polymer chains, particularly their length, independent of the graft density. If, e.g., the polymerization is carried out in a solvent where the monomer concentrations can be controlled, a higher monomer concentration will directly lead to a higher molecular weight of the resulting polymers.

According to this precise control of the parameters graft density and molecular weight, it is possible to adapt the properties of the respective polymer layers to a variety of applications. For example, layers of different thickness can be produced over a wide range from a few nanometers up to a few micrometers. It is also possible to fine-tune the properties of the resulting layer, e.g. with respect to the accessibility of the functional groups for subsequently coupled probe and sample molecules which may vary considerably in their size and structure.

The polymer chains obtained via the above preferred method retain a fragment of the initiator in their structure which immobilizes them on the surface, namely the portion starting with the anchoring site and leading to the the predetermined point of initiation as it is known in the art for all types of initiators, in particular those mentioned in this application.

Detailed information on the synthesis of initiator molecules, their reaction with surfaces and the preferred conditions of polymerization are described in:

- O. Prucker, J. Rühe, Macromolecules, 1998,31, 592;
- O. Prucker, J. Rühe, Macromolecules, 1998, 31, 602 and
- O. Prucker, J. Rühe, Langmuir, 1998, 24 (14), 6893.

Care should be taken to remove unreacted monomers as well as non-bonded polymer chains with suitable solvents after polymerization.

Polymer layers prepared according to this method can be applied to a wide variety of surfaces, independent of their shape. Even surfaces which are inaccessible for conventional surface modification methods (e.g. inner surfaces) can be provided with the polymer monolayers according to the invention, since no bulky polymer molecules have to diffuse towards the surface.

Also, it is possible to create patterned arrays of the polymer monolayers by various means. One way are standard photolithographic processes that can either be applied after polymerization (photoablation of the polymers through masks) prior to this step (photodecomposition or photoablation of the initiator monolayer masks) or during the polymerization by means of photopolymerization through masks. Other possible techniques for the creation of patterned polymer monolayers are microcontact printing or related methods, which may be applied during formation of the initiator layer or during polymerization. Finally, ink jet techniques or other microplotting methods can be used to create patterned initiator monolayers which can subsequently be transferred to patterned polymer monolayers. Using any of these techniques, surface structures with dimensions in the micrometer range can be created. The high parallel mode of signal generation and a significant improvement in the integration of analytical data is the most promising feature of such techniques, which accordingly allow the optimization of automatic analytical procedures.

For the detection of a successful immobilization of sample or probe molecules on a polymer monolayer, a variety of techniques can be applied. In particular, it has been found that the polymer layers of the present invention undergo a significant increase in their thickness which can be detected with suitable methods, e.g. ellipsometry. Mass sensitive methods may also be applied.

If nucleic acids, for example oligonucleotides with a desired nucleotide sequence or DNA molecules in a biological sample, are to be analyzed, synthetic

oligonucleotide single strands can be reacted with the polymer monolayer. The reaction is carried out under high humidity, preferably in a buffered aqueous solution. The reaction temperature can be raised above room temperature, as long as it is not detrimental to the oligonucleotides. Preferred temperatures are in the range of 40-60°C. In this application, a multitude of identical synthetic oligonucleotide strands or a mixture of different strands can be used. If different strands are used, their sequences should preferably be known.

Before the thus prepared surface is used in a hybridization reaction, unreacted functional groups are deactivated via addition of suitable nucleophiles, preferably C_1-C_4 amines, such as simple primary alkylamines (e.g. propyl or butyl amine), secondary amines (diethylamine) or amino acids (glycin).

Upon exposure to a mixture of oligonucleotide single strands, e.g. as obtained from PCR, which are labled, only those surface areas which provide synthetic strands as probes complementary to the PCR product will show a detectable signal upon scanning due to hybridization. In order to facilitate the parallel detection of different oligonucleotide sequences, printing techniques can be used which allow the separation of the sensor surface into areas where different types of synthetic oligonucleotide probes are presented to the test solution.

The term "hybridization" as used in accordance with the present invention may relate to stringent or non-stringent conditions. If not further specified, the conditions are preferably non-stringent. Said hybridization conditions may be established according to conventional protocols described, for example, in Sambrook, "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (1989), Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989), or Higgins and Hames (Eds) "Nucleic acid hybridization, a practical approach" IRL Press Oxford, Washington DC, (1985). The setting of conditions is well within the skill of the artisan and to be determined according to protocols described in the art. Thus, the detection of only specifically hybridizing sequences will usually require stringent hybridization and washing conditions such as for example 0.1xSSC, 0.1% SDS at 65°C. Exemplary non-stringent hybridization conditions for the detection of homologous or not exactly complementary sequences may be set at 6xSSC, 1% SDS at 65°C. As is well known, the length of the probe and the composition of the

nucleic acid to be determined constitute further parameters of the hybridization conditions.

The nucleic acids to be analyzed may originate from a DNA library or a genomic library, including synthetic and semisynthetic nucleic acid libraries. Preferably, the nucleic acid library comprises oligonucleotides.

In order to facilitate their detection in an immobilized state, the nucleic acid molecules should preferably be labeled. Suitable labels include radioactive, fluorescent, phosphorescent, bioluminescent or chemoluminescent labels, an enzyme, an antibody or a functional fragment or functional derivative thereof, biotin, avidin or streptavidin.

Antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric or single chain antibodies or functional fragments or derivatives of such antibodies.

The general methodology for producing antibodies is well-known and has been described in, for example, Köhler and Milstein, Nature 256 (1975), 494 and reviewed in J.G.R. Hurrel, ed., "Monoclonal Hybridoma Antibodies: Techniques and Applications", CRC Press Inc., Boco Raron, FL (1982), as well as that taught by L. T. Mimms et al., Virology 176 (1990), 604-619. As stated above, in accordance with the present invention the term "antibody" relates to monoclonal or polyclonal antibodies. Functional antibody fragments or derivatives provide the same specificity as the original antibody and comprise $F(ab')_2$, Fab, Fv or scFv fragments; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual", CSH Press 1988, Cold Spring Harbor, NY. Preferably the antibody of the invention is a monoclonal antibody. Furthermore, in accordance with the present invention, the derivatives can be produced by peptidomimetics. Such production methods are well known in the art and can be applied by the person skilled in the art without further ado.

Depending on the labeling method applied, the detection can be effected by methods known in the art, e.g. via laser scanning or use of CCD cameras.

Also comprised by the present invention are methods where detection is indirectly effected. An example of such an indirect detection is the use of a secondary labeled antibody directed to a first compound such as an antibody which binds to the biological molecule (sample molecule) of interest.

A further application of the polymer monolayers according to the invention lies in the field of affinity chromatography, e.g. for the purification of substances. For this purpose, polymer brushes with identical functional groups or probe molecules are preferably used, which are contacted with a sample. After the desired substance has been immobilized by the polymer brush, unbound material can be removed, e.g. in a washing step. With suitable eluents, the purified substance can then be separated from the affinity matrix.

Preferred substances which may be immobilized on such a matrix are nucleic acid molecules, peptides or polypeptides (or complexes thereof, such as antibodies, functional fragments or derivatives thereof), saccharides or polysaccharides.

A regeneration of the surfaces after the immobilization has taken place is possible, but single uses are preferred in order to ensure the quality of results.

With the present invention, different types of samples can be analyzed with an increased precision and/or reduced need of space in serial as well as parallel detection methods. The sensor surfaces according to the invention can therefore serve in diagnostical instruments or other medical applications, e.g. for the detection of components in physiological fluids, such as blood, serum, sputum etc.

Surfaces according to the present invention can also immobilize starter molecules for synthetic applications in particular in solid phase synthesis, e.g. during the in situ formation of oligo- or polymers. Preferably, the oligo- or polymers are biomolecules and comprise peptides, proteins, oligo- or polysaccharides or oligo- or polynucleic acids. As immobilized initiators, a monomer of these macromolecules can be used.

Moreover, the polymer layers of the present invention can be used as gels in the separation of molecules, preferably biomolecules in an electrical field.

Generally, the present invention allows the provision of homogenically modified surfaces with superior graft density. By choosing the appropriate polymerization conditions, the graft density and the chain length, and thus the thickness of the polymer layer can be controlled. Moreover, structured surfaces can be provided, e.g. by starting the polymerization from patterned arrays of initiator molecules. As a consequence, the polymer monolayers can be adjusted optimally to the respective applications.

The disclosure content of the documents cited throughout the specification are herewith incorporated by reference.

The embodiments of the present invention are further illustrated in the following items:

A preferred process for the detection of sample nucleic acid molecules, preferably of single stranded nucleic acid molecules, using a polymer layer according to the invention comprises the steps of:

a) providing a surface covered with a polyfunctional polymer monolayer according to the invention
b) immobilizing suitable probe molecules, preferably oligonucleotide single strands on the polymer monolayer via a reaction with the functional groups present in the polymer chains
c) allowing a hybridization reaction to take place between the oligonucleotide single strands and the sample nucleic acid molecules,
d) removal of the non-hybridized nucleic acid molecules in a washing step and
e) detection of the hybridized nucleic acid molecules, preferably fluorometric.

A preferred process for purifying a compound from a sample, using a polymer layer according to the invention comprises the steps of :

a) providing a surface modified with a polymer monolayer according to the invention
b) immobilizing a multitude of identical probe molecules on the polymer layer
c) contacting the sample with the resulting polymer layer, under conditions that allow binding of said compound to the probe molecule;

d) and removing material from the sample that has not bound to the probe molecule.

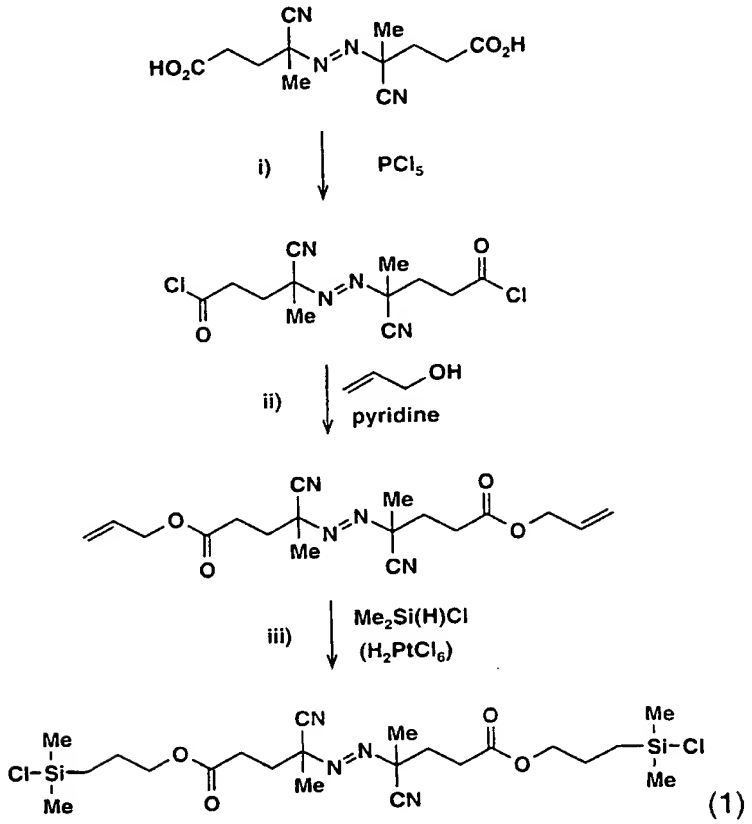
This process may further include the step of

e) separating the compound from the probe molecule by use of a suitable eluent.

The following examples illustrate the invention:

(1) Synthesis of the initiator

As an example, the preparation of compound 1 is described. The reaction pathway is illustrated below. The indices i-iii in the Figure refer to the description of the various steps in the text.



(1)

i) To a suspension of 40 g phosphorus pentachloride (PCl_5) in 50 ml methylene chloride cooled with an ice-bath was added dropwise a suspension of 10 g of 4,4'-azobis-(4-cyano pentanoic acid) in 50 ml methylene chloride. The mixture was allowed to warm to room temperature and stirred overnight. The excess PCl_5 was filtered off and the remaining solution was concentrated until no more PCl_5 separated. The mixture was filtered again and the filtrate was added to 300 ml of cold hexane, causing the separation of the acid chloride as a white solid (yield: 90%).

ii) To a solution of 2.7 ml of allyl alcohol and 6.5 ml of pyridine in 50 ml methylene chloride at 0°C was added dropwise a solution of 10 g of the acid chloride in 50 ml methylene chloride. The mixture was allowed to warm to room temperature and stirred overnight. Then the solution was washed twice with 2N H_2SO_4, aqueous $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The resulting bis allylic ester was recrystallized from methanol (yield: 90%).

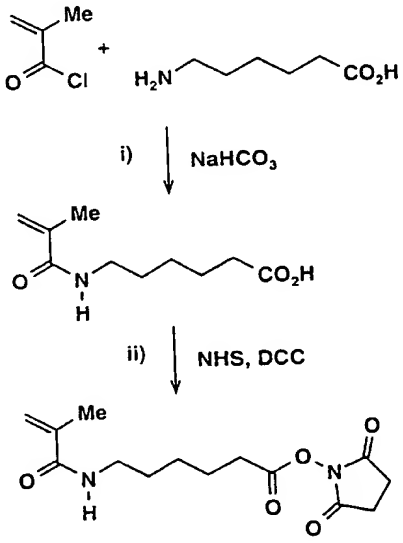
iii) To a suspension of 3 g of the bis allylic ester in 30 ml dimethyl chloro silane was added a solution of 30 mg of hexachloroplatinic acid in 0.5 ml of dimethyl ether/ethanol (1/1 v/v), and the mixture was heated to reflux for 3 h. The excess of the silane was evaporated yielding compound 1 as a pale green oil in quantitative yields. Residual platinum catalyst was removed by filtration of a methylene chloride solution of the product over anhydrous Na_2SO_4.

(2) Formation of an initiator monolayer

The initiator synthesized under (1) is immobilized at room temperature on a glass surface under inert conditions (atmosphere of dry nitrogen) using anhydrous toluene as a solvent and dry triethylamine as catalyst. The toluene solution shows a concentration of the initiator of about 50 mmol/l, triethylamine is added up to a concentration of about 10 mmol/l. The samples are kept in the solution overnight and then cleaned by extensive rinsing with methanol and chloroform.

(3) Synthesis of the functionalized monomer

As an example, the synthesis of N-methacryloyl-6-aminocapronic acid hydroxysuccinimide ester is described. The reaction pathway is shown below. The indices i-iii in this Figure refer to the description of the various steps in the text.



i) A solution of 13.2 g 6-aminocaproic acid and 20 g $NaHCO_3$ in 100 ml water and 50 ml 1,4-dioxane was slowly added to a solution of 10.3 ml of methacrylic acid chloride in 50 ml 1,4-dioxane. The solution was stirred overnight. Then 50 ml of water were added and the mixture was washed three times with 100 ml portions of ethyl acetate. The water layer was acidified (pH 2) with dilute hydrochloric acid and then extracted with three 100 ml portions of ethyl acetate. The combined organic layers were dried over Na_2SO_4, concentrated to a volume of about 50 ml and added to 350 ml of cold hexane. This mixture was cooled to -20°C, and the product slowly separated overnight as white crystals (yield: ca. 14 g).

ii) A solution of 14 g of the acid in 300 ml methylene chloride was cooled to 5°C and 8.2 g of N-hydroxy succinimide (NHS) and 14.6 g of N,N-dicyclohexyl carbodiimide were added. The mixture was kept at 5°C overnight. The precipitate (dicyclohexylurea) was filtered off and the solvent was evaporated. During this step, additional urea separated in some cases and was also filtered off. The crude

product was recrystallized from isopropanol to yield about 15 g of the NHS ester monomer.

(4) Formation of a polyfunctional polymer monolayer

A comonomer mixture of N,N-dimethyl-acrylamide (DMAA) and N-methacryloyl-6-aminocapronic acid hydroxy succinimide ester (C_6AE) obtained from (3) is polymerized in dimethylformamide (DMF) as solvent. The monomer concentration is 4 mol/l at a molar ratio of the comonomers of DMAA/C_6AE=5/1. The polymerization is performed at 60°C. Prior to polymerization, the solutions are carefully degassed through at least 3 freeze-thaw-cycles in order to remove all oxygen traces. After polymerization, every sample is extracted with DMF for at least 10 hours.

(5) Detection of oligonucleotides strands

The obtained surface is exposed to 1 nl of a 10 μM oligonucleotide-solution and the coupling reaction is allowed to proceed at about 40-50°C for two hours in an aqueous solution.

The synthetic oligonucleotide is 5-amino modified, and the solution is buffered with a 100 mM sodium phosphate buffer at a pH of 8.0. After the coupling reaction, the sensor surface is rinsed with the sodium phosphate buffer. In order to define the spatial extension of the specific types of oligonucleotide on the sensor surface for parallel detection, the reactant was printed onto the polymer layer.

The surface thus prepared was allowed to react with a Cy5 labeled PCR product in a buffer of 2xSSC, 10% dextrane sulphate and 50% formamide for 12h at 28°C. The DNA content was 100 ng DNA /80 μl sample. After the hybridization reaction has taken place, the surface was washed in SSC-buffer and the result was detected fluorometrically via laser activation with a CCD camera. A fluorescence signal could only be detected for those areas which carried synthetic oligonucleotides complementary with the PCR product.

Claims

1. Polyfunctional polymer monolayer comprising an assembly of polymer chains attached to a surface, characterized in that each polymer chain comprises a multitude of identical or different units carrying one or more functional groups which allow an interaction of the polymer with a sample or probe molecule.

2. Polymer monolayer according to claim 1, wherein the polymer chains are covalently attached to the surface.

3. Polymer monolayer according to claim 1 or 2, wherein the functional groups are chosen from carboxylic acids, maleinimides, N-hydroxy succinimides, epoxides, isothiocyanates, isocyanates or azides.

4. Polymer monolayer according to any of claims 1 to 3, wherein the sample molecule or probe molecule is chosen from proteins, peptides, polysaccharides or nucleic acids and derivatives thereof.

5. Polymer monolayer according to any of claims 1 to 4, wherein the polymer comprises segments that make the layer water swellable.

6. Polymer monolayer according to claim 5, wherein the water swellability is provided by monomers chosen from acrylic acid, methacrylic acid, dimethyl acrylamide or vinyl pyrrolidon.

7. Polymer monolayer according to any of claims 1 to 3, 5 and 6, further comprising a multitude of identical or different probe molecules immobilized at the polymer chain via a reaction with the functional groups.

8. Polymer monolayer according to claim 7, wherein the probe molecules are selected from nucleic acids, PNAs, polysaccharides, proteins and peptides.

9. Surface carrying a polyfunctional polymer monolayer according to any of claims 1 to 8.

10. Surface according to claim 9, wherein the polymer chains are in the form of patterned arrays.

11. Process for the production of a polyfunctional polymer monolayer according to any of claims 1 to 8, comprising the steps of:

a) covering the surface with a monolayer of a polymerization initiator which comprises one or more functional groups suitable for attachment to the surface; and

b) initiating and carrying out a polymerization reaction in the presence of monomers carrying functional groups which allow a coupling reaction of the obtained polymer chain with specific sample molecules.

12. Process according to claim 11, wherein the initiator comprises a chlorosilane, an alkoxysilane, a disulphide or a thiol group.

13. Process according to claims 11 or 12 wherein the initiator comprises a group chosen from azo groups, peroxo groups, or a ketone group in conjugation with an aromatic system.

14. Process according to claim 13, wherein the initiator comprises a group chosen from aromatic ketones or aromatic ketones containing sulphur.

15. Process for the detection of sample nucleic acid molecules, using a polymer monolayer according to claim 7 or 8, which comprises the steps of

a) allowing a hybridization reaction to take place between the probe and the sample, followed by
b) removal of the non hybridized nucleic acid molecules in a washing step and
c) detection of the hybridized nucleic acid molecules.

16. A process for purifying a compound from a sample comprising the steps of

    (a) contacting the sample with the polymer monolayer of any of claims 1 to 8, under conditions that allow binding of said compound to the functional group of the polymer chain or the probe molecule;
    (b) and removing material from the sample that has not bound to the polymer layer or a probe molecule;

17. The process according to claim 16 further comprising

    (c) eluting the bound complex from the polymer layer.

18. The process according to claim 16 or 17, wherein said compound is a nucleic acid, a (poly)saccharide or a (poly)peptide or a complex thereof, preferably an antibody or a fragment or derivative thereof.

19. Use of the surface according to claims 9 or 10 as an affinity matrix.

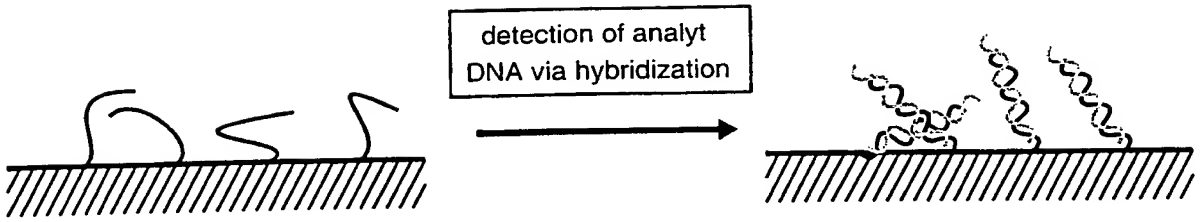
20. Use of a surface according to claims 9 or 10 in a sensor chip.

21. Medical or diagnostic instrument, comprising a surface according to claims 9 or 10.

22. Use of a surface according to claims 9 or 10 for the immobilization of starter molecules for the formation of oligo- or polymers, preferably for nucleic acid or peptide synthesis.

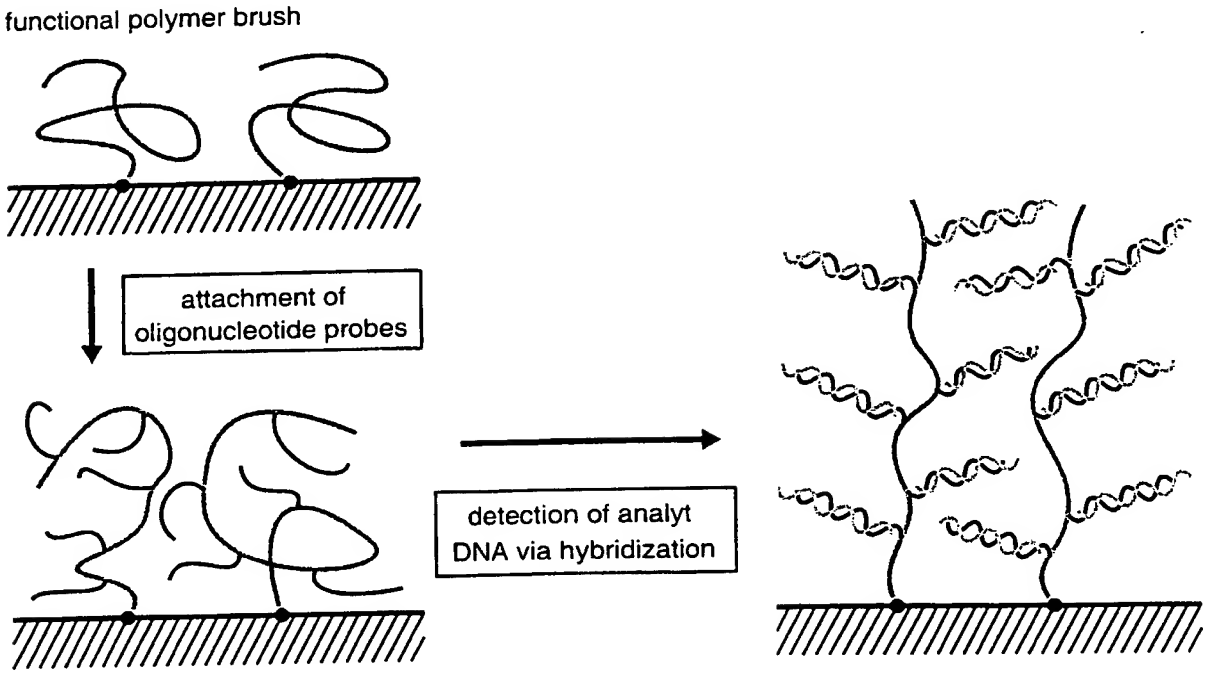
23. Use of polymer layer according to any of claims 1 to 8 as a gel in the separation of molecules in an electric field.

Fig. 1



immobilized oligonucleotide strands

Fig. 2



polymer brush with oligonucleotide strands (actual sensor device)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: **BIOCHIP TECHNOLOGIES GMBH** [DE/DE]; Engesserstrasse 4b, D-79108 Freiburg im Breisgau (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KLAPPROTH, Holger** [DE/DE]; Kehlerstrasse 12, D-79108 Freiburg (DE). **PRUCKER, Oswald** [DE/DE]; Sertoriusring 283, D-55126 Mainz (DE). **RÜHE, Jürgen** [DE/DE]; Sattlerweg 9a, D-55128 Mainz (DE).

(74) Agent: **VOSSIUS & PARTNER**; Siebertsrasse 4, D-81675 München (DE).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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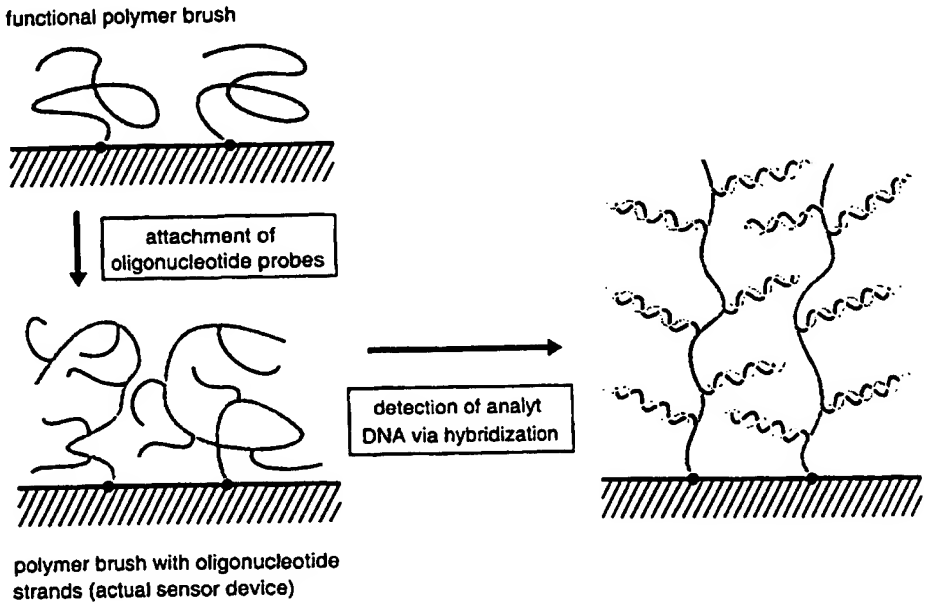
(54) Title: IMMOBILIZATION OF MOLECULES ON SURFACES VIA POLYMER BRUSHES



(57) Abstract: The invention relates to polyfunctional polymer monolayers (polymer brushes) comprising a multitude of polymer chains attached to a surface, with each polymer chain comprising a multitude of units carrying at least on functional group which allows the interaction of the polymer chain with a sample molecule.

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(88) **Date of publication of the international search report:**
30 November 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Inter[illegible]pplication No
PCT/EP 00/00554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 B01J19/00 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q B01J G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 41425 A (PENCE INC ;UNIV MCGILL (CA)) 6 November 1997 (1997-11-06) see figures and claims ---	1-23
X	WO 90 05303 A (PHARMACIA AB) 17 May 1990 (1990-05-17) see whole doc., esp. claims and p.8, 2-par. ff ---	1-23
X	SÖNKSEN C.P. ET AL.,: "Combining Maldi mass spectrometry and biomolecular interaction analysis using a biomolecular interaction analysis instrument" ANAL. CHEM., vol. 70, - 1 July 1998 (1998-07-01) pages 2731-2736, XP002111582 the whole document --- -/--	16-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THIEL A J ET AL: "IN SITU SURFACE PLASMON RESONANCE IMAGING DETECTION OF DNA HYBRIDIZATION TO OLIGONUCLEOTIDE ARRAYS ON GOLD SURFACES" ANALYTICAL CHEMISTRY, vol. 69, no. 24, 15 December 1997 (1997-12-15), pages 4948-4956, XP000733394 ISSN: 0003-2700 the whole document ---	
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A	RIBBE A. ET AL.,: "Imaging of polymer monolayers attached to silica surfaces by element specific transmission electron microscopy" POLYMER, vol. 37, no. 7, - 1996 pages 1087-1093, XP002111583 the whole document -----	

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information on patent family members

Inter. [illegible] Application No
PCT/EP 00/00554

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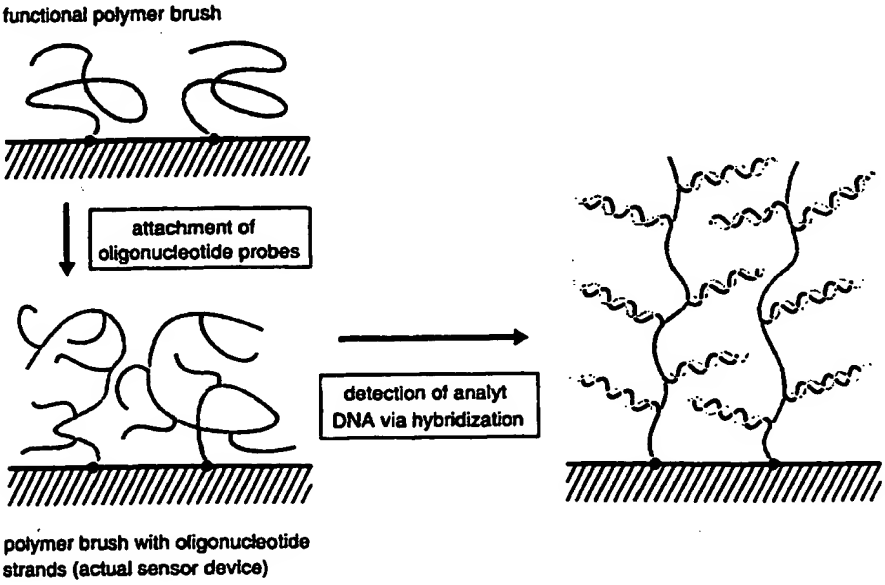

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Inter [redacted] Application No
PCT/[redacted]00/00554

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B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

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INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

Inter. [redacted] Application No
PCT/[redacted]00/00554

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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